Influence of pH value on sulfonamide ozonation using caffeine as a contamination indicator

Glenda Araujo S. Gireli, Milena Guedes Maniero and José Roberto Guimarães

ABSTRACT

Ozonation was effective in degrading six sulfonamides: sulfaquinoxaline, sulfamethazine, sulfamethoxazole, sulfathiazole, sulfadimethoxine, and sulfadiazine ($C_0 = 100\ \mu g\ L^{-1}$). The degradation experiments were based on two variables: pH (3.0, 7.0 and 11.0) and aqueous matrix (ultrapure water, tap water, and surface water). In all matrices studied and pH 11.0, more than 99% of the drugs were degraded with the highest applied ozone dose (65 mg L^{-1}), when compared with other pH values. Also, comparing the three aqueous matrices, the ultrapure water was the one that required the lowest ozone dosage (6.4 mg L^{-1}) to reach the same degradation efficiency of the sulfonamides. Caffeine is known as an environment contamination indicator. Therefore, this does not intend to totally remove this compound, but to verify its degradation level compared with the sulfonamides.

Key words | advanced oxidation process, antimicrobial activity, drinking water, superficial water, tap water, toxicity assays

Glenda Araujo S. Gireli
Milena Guedes Maniero
José Roberto Guimarães (corresponding author)
School of Civil Engineering, Architecture and Urban Design,
University of Campinas,
P.O. Box 6143, 13083-889 Campinas,
Brazil
E-mail: *jorober.fec@gmail.com*

INTRODUCTION

The use of antibiotics has recently become a growing and unavoidable practice. While this has improved human and animal welfare, on the other hand, the presence of residues of these drugs has become a source of concern for the international scientific community.

However, due to inefficient public policies, disorganized population growth and precarious sanitation services, the quality of water resources has deteriorated more and more. Added to this is the daily and increasing release of chemical pollutants into the environment.

Many of these pollutants are now designated contaminants of emerging concern, since they are constantly detected in aquatic environments, water and sewage treatment plants and because the impact of these compounds on human health is not fully understood.

Over the past few years, a class of compounds of emerging concern has drawn the attention of the scientific community: sulfonamides (Urbano *et al.* 2017). Since sulfonamides are persistent compounds, their residues have constantly been detected in aquatic environments, wastewater treatment plants (WWTP) and drinking water treatment plants (DWTP) (Petrie *et al.* 2015). Therefore, studies have been showing the presence of antibiotic residues in food products, increasing the potential risk to consumers in terms of carcinogenicity and allergic reactions, and contributing to the development of bacterial resistance (FAO 2016).

Sulfonamides have been detected in groundwater (García-Galán *et al.* 2011), drinking water (Wu *et al.* 2018) and in wastewater (Kim *et al.* 2013). Although the concentration of drugs in the aquatic environment is relatively low (ng L^{-1} to $\mu g\ L^{-1}$), their biological activity is high, even in small amounts, and it can cause significant changes in the biosphere.

doi: 10.2166/ws.2019.182

To prevent the pollution of the aquatic environment, an effective elimination of sulfonamides from wastewater must occur. Over the last 10 years, several conventional treatments have been tested for the removal of antibiotics from environmental matrices (Homem & Santos 2011).

Gobel *et al.* (2007) evaluated the traditional primary and secondary treatments used in WWTP in matrices containing macrolides, sulfonamides and trimethoprim. In this case, low elimination rates of 20% were observed.

Among these alternatives, advanced oxidation processes (AOPs) are considered highly effective (Kim *et al.* 2013) in relation to traditional water treatment processes due to their ability to degrade toxic and poorly biodegradable organic pollutants. AOPs involve the generation of hydroxyl radicals (•OH), a highly oxidizing and non-selective compound with high reduction potential ($E_0 = 2.73$ V/SHE). Many studies have applied AOPs for sulfonamide degradation (Lin *et al.* 2009; Garoma *et al.* 2010; Xu *et al.* 2010; Sui *et al.* 2011; Won *et al.* 2014; Wu *et al.* 2015; Urbano *et al.* 2017; Yu *et al.* 2017).

Ozonation has been quite efficient in water treatment, either as an alternative to conventional processes or as a complementary treatment. The pH value can also be a determining factor in its mechanism of action, since, under acidic conditions, direct oxidation with molecular ozone is predominant; and at high pH values, conditions are favorable for the generation of hydroxyl radicals, where the indirect form of oxidation is predominant since ozone decomposes into hydroxyl radicals (EPA 1999).

Due to the large number of compounds of emerging concern and the new ones being introduced every year in society, it is difficult for legislation on water quality to encompass all of them. One way to verify if these compounds are being properly disposed of in wastewater treatment and effluents is to use a chemical compound as a contamination indicator. Thus, if there is any indication that even after sewage and water treatment there are still traces of this compound, many others are likely to appear as well. Caffeine, for example, is an indicator that can be quickly detected, as it is present in high concentrations in the sewage for being one of the most consumed products in the world, besides being a stable compound and very soluble in water. Due to these characteristics, studies have been investigating caffeine as a contamination indicator (Paíga & Delerue-Matos 2017).

Despite the good results obtained with the use of ozonation to degrade sulfonamides, the literature lacks information about the degradation of six sulfonamides at the same time in different matrices using caffeine as a degradation indicator.

Thus, this study aims to contribute to the scientific community by investigating the degradation of six sulfonamides ($C_0 = 100\ \mu g\ L^{-1}$) (sulfaquinoxaline (SQX), sulfamethazine (SMZ), sulfamethoxazole (SMX), sulfathiazole (STZ), sulfadimethoxine (SDM), and sulfadiazine (SDZ)) by ozonation in three types of water matrices (ultrapure water (UPW), tap water (TW) and surface water (SW)) using caffeine as a contamination indicator, and in three pH conditions. Also, two acute toxicity tests (using the marine *Vibrio fischeri* bacterium and the microcrustacean *Daphnia similis*) promoted by ozonation along with the removal of the antimicrobial activity against Gram-positive (*Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*) was evaluated.

To perform one of the acute toxicity assays, the aquatic invertebrate *Daphnia similis* was used, a microcrustacean bioindicator commonly used as a test organism to assess the ecotoxicity of chemicals (Dal Bosco *et al.* 2011; Guimarães *et al.* 2014).

METHODS

Chemicals and microorganisms

All reagents used in this study were of analytical or pure grade. Analytical sulfadiazine standards (SDZ; 99% purity; $C_{10}H_{10}N_4O_2S$; 250.28 g mol^{-1}), sulfathiazole (STZ; 98% purity $C_9H_9N_3O_2S_2$; 255.32 g mol^{-1}), sulfamethazine (SMZ; 99% purity; $C_{12}H_{14}N_4O_2S$; 278.33 g mol^{-1}), sulfamethoxazole (SMX; 99% purity; $C_{10}H_{11}N_3O_3S$; 253.28 g mol^{-1}), sulfaquinoxaline (SQX; 95% purity; $C_{14}H_{12}N_4O_2S$; 300.30 g mol^{-1}), sulfadimethoxine (SDM; 99% purity; $C_{12}H_{14}N_4O_4S$; 310.30 g mol^{-1}), and caffeine (CAF; 99% purity; $C_8H_{10}N_4O_2$; 194.2 g mol^{-1}) were obtained from Sigma-Aldrich (USA). Formic acid (98%–100%, CH_2O_2) was obtained from Merck, hydrogen peroxide (30% m/m, H_2O_2) and concentrated sulfuric acid (97%, H_2SO_4) from Synth, sodium hydroxide (97% NaOH) from Ecibra, and HPLC grade methanol (CH_3OH) from JT Baker. Working solutions of sulfonamides were prepared in 1 L of the analyzed aqueous matrix. Ultrapure water

was obtained from the Millipore Milli-Q purification system. The characterization of the tap water (from a DWTP) was obtained from the website of the company responsible for water and wastewater treatment of Campinas city (SANASA) (Table S1, Supplementary Material). Also, the tap water underwent a boiling process to remove chlorine. The surface water was obtained from Chico Mendes Lake, located in the Ecological Park Prof. Hermógenes de Freitas Leitão (Campinas, São Paulo, Brazil). For the surface water matrix, the following parameters were measured: DO, hardness, alkalinity, pH, conductivity, color and turbidity (Table S2).

Antimicrobial assays were performed with Gram-negative bacteria *Escherichia coli* ATCC® 23716 and Gram-positive bacteria *Bacillus subtilis* ATCC® 168. These strains were obtained from ATCC (Manassas, USA) and CPQBA (Campinas city, Brazil), respectively. *Vibrio fischeri* strains purchased from Unwelt (Blumenal city, Brazil) were used in the Microtox® toxicity assays.

The initial concentration of each sulfonamide was 100 μg L^{-1}, and the solution was prepared at the time of the assay. Different initial pH values (3.0, 7.0 and 11.0) were used to evaluate the degradation of sulfonamides. At the end of the ozonation, the pH values of the solutions were all adjusted to 7 before mass spectrometry analysis and toxicity assays. Bench-scale ozonation tests were performed using the O3R Philozon ozone generator and a contact column of 1 L (50 cm height and 7 cm inner diameter). The ozone generator produced an average of 12.1 mg L^{-1} min^{-1} O_3, using air (flow rate of 4.0 L min^{-1}).

The ozone was introduced into the reactor by a sintered glass diffuser. The O_3 concentration at the inlet and outlet of the reactor was quantified by the iodometric method – 2350E from *Standard Methods for the Examination of Water and Wastewater* (APHA 2012), using a solution of potassium iodide (KI). The ozone dose applied was obtained by multiplying the ozonation time (0–5 min) by the ozone generation (average of 12.1 mg L^{-1} min^{-1} O_3), and then dividing by the sample volume (van Leeuwen 2015). After the ozonation, the samples were flushed with nitrogen for 5 min to remove residual ozone dissolved in the aqueous phase.

Analytical methods

The analytes were quantified using online solid phase extraction (SPE) coupled to ultra-high-performance liquid chromatography with mass spectrometry (SPE-UHPLC-MS/MS). This system was obtained from Water (USA) and equipped with an electrospray ionization source (ESI) and triple quadrupole (Waters® Xevo® TQD Acquity UPLC), operated in positive ionization mode.

An OASIS HLB column (2.1 × 30 mm, 20 μm) was used for on-line SPE. Analytes were separated into an ACQUITY BEH C18 UPLC analytical column (50 mm × 2.1 mm, 1.7 μm, Waters, Ireland). The mobile phases were water with 0.2% formic acid and methanol, in isocratic mode (40:60, v/v) at a flow rate of 0.35 mL min^{-1}.

Acute toxicity assays using *Vibrio fischeri*

The toxicity test was based on the inhibition of luminescence of the marine bacteria *Vibrio fischeri* in the initial and ozonated solutions. The assays were performed in duplicate and they were based on the CETESB L5.227 standard protocol using a Microtox Model 500 analyzer (Modern Water Inc., Newark, DE, USA) and the Microtox 81.9% Basic Test. Data were treated using Omni 4.2 software (Modern Water Inc.). The contact time was 30 min. The evolution of toxicity was expressed in percentage of luminescence inhibition (%), following the established Microtox protocol.

Acute toxicity assays using *Daphnia similis*

These acute toxicity assays were based on the NBR 12713 (ABNT 2009) Brazilian standard. Young *Daphnia similis* organisms were exposed to the six sulfonamide solutions untreated and degraded by the ozonation process, at different times of degradation to evaluate the toxicity reduction during the AOPs. These assays were performed in duplicate.

Initially, a qualitative assay was performed with undiluted samples. No statistically significant immobility was found in relation to the control, even with the initial concentration without degradation, and it was not necessary to dilute the samples with *Daphnia similis* cultivation water. Also, as this was a qualitative analysis, we verified that it would not be possible to perform with the ultrapure water matrix, since it does not have the necessary conditions for survival of *Daphnia similis*, and if the same matrix were diluted with diluted water, the results could be masked by the conditions of this matrix.

Antimicrobial activity assays

Antimicrobial activity assays were quantified in duplicate and performed according to the method described by Caianelo *et al.* (2017), with some modifications, as the samples were not serially diluted.

The bacteria *Bacillus subtilis* and *Escherichia coli* culture sample solution (in Mueller–Hinton broth) was thawed at 25 °C and 0.1 mL was inoculated into 10 mL of Mueller–Hinton culture broth. The culture was then incubated for 24 h at 37 °C and then diluted to match the absorbance of a McFarland stock solution (0.5 mL of 0.048 mol L^{-1} $BaCl_2$ in 99.5 mL of 0.18 mol L^{-1} H_2SO_4) at 625 nm. This absorbance corresponded to a population density of 1.0×10^8 CFU mL^{-1}. The antimicrobial activity was evaluated using the bacterial culture at a population density of 1.0×10^6 CFU mL^{-1}.

An aliquot of each sample (80 μL) was added to two wells of a 96-well microtiter plate, and 20 μL of 1 mmol L^{-1} phosphate buffer (pH 8) was added to the sample. One well contained only the buffer solution (100 μL) to verify the bacterial growth in the absence of any sample (controls). After that, all the wells (with samples and controls) were inoculated with 100 μL of the bacteria culture (*E. coli* or *B. subtilis*). The plate was sealed and incubated for 8 h at 37 °C. After this period, the absorbance of each sample well was measured at 620 nm by a SpectraMax microplate reader.

RESULTS AND DISCUSSION

For all the matrices studied in this work, applied and consumed ozone were verified at each pH analyzed (3.0, 7.0 and 11.0). The consumed ozone concentration was the difference between the ozone dosage at the inlet and outlet of the bubble column, measured using the KI solution, and varied according to the pH used and the ozonation time (between 0 and 5 min). The portion of the applied dose will react immediately; this is called consumed ozone. Results are shown in Tables S3, S4 and S5 (Supplementary Material). Thus, as the complexity of the matrix increases, so does the amount of consumed ozone in the degradation process of the sulfonamides present.

Ozonation at different pH values (3.0, 7.0 and 11.0) was carried out to compare the degradation of the six sulfonamides in the three types of water matrices using caffeine as a degradation indicator. The results indicated a positive correlation between two variables: applied ozone dosage and degradation efficiency. This means that a higher applied ozone dose resulted in a greater degradation of the sulfonamides.

Caffeine proved to be an excellent molecule to assess the level of sulfonamide degradation by the ozonation process, since it was the last compound to be degraded. The results using ultrapure water as matrix show that, at pH 3.0 (Figure S1), for a >99% degradation of the sulfonamides, only 20.1% of the caffeine had been degraded with an applied ozone dosage of 6.3 mg L^{-1}.

For pH 7.0, to obtain the same percentage as at pH 3.0 degradation, 10.9 mg L^{-1} of applied ozone was required, and, for that value, only 11.9% of the caffeine was degraded. And, finally, at pH 11.0, once 8.9% of the caffeine was degraded, it was possible to obtain an elimination rate of >96% of the sulfonamides evaluated in this study, which was achievable by applying an ozone dose of 53.2 mg L^{-1}.

Urbano *et al.* (2017) verified that an applied ozone dosage of 5.5 mg L^{-1} was required for similar degradation. However, in this study, six sulfonamides were used simultaneously, which could explain the greater need of ozone dose. At pH 11.0, the degradation efficiencies, applying for the same ozone dosage, were: 47%, 92%, 44%, 37% 38%, and 30% for SDA, STZ, SMZ, SMX, SDM, and SQX, respectively. Those results can be seen in the Supplementary Material.

In the study by Garoma *et al.* (2010), despite working with an initial concentration of sulfonamides 10 times greater (1,000 μg L^{-1}), more than 90% removals of the drugs were achieved. The authors observed that by increasing the pH from 2.0 to 10.0 the removal of sulfadiazine, sulfamethazol, sulfamethoxazole and sulfathiazole also increased. However, in this study, the sulfonamide degradation efficiency was higher for lower pH values.

Direct oxidation through molecular ozone promoted a greater degradation of the studied drugs. The molecular ozone has high selectivity, so, at pH 3.0, it would mainly attack the sulfonamide molecules, whereas at pH 11.0 the hydroxyl radicals would attack both the molecules and their degradation products.

Lin *et al.* (2009) also reported that the higher the pH, the lower the sulfonamide degradation by ozonation. According to the authors, this occurred for the following reasons: low pH results in low OH^- concentrations, which allows a

greater accumulation of dissolved O_3 due to the low depletion reaction, whereas in high pH this reaction is faster because OH^- concentrations are abundant, which prevents the accumulation of dissolved O_3 (Lin *et al.* 2009; Sui *et al.* 2011). Thus, the degradation of compounds susceptible to electrophilic attack by O_3 is more rapid at low pH, where aqueous O_3 is higher.

The results obtained for the ultrapure water matrix can be compared with some studies on traditional treatments and other advanced oxidative processes for sulfonamide degradation in this matrix. For example, in the study by Guo *et al.* (2016) UV light, ozone and the two combined processes for the removal of sulfadiazine were used. The UV light removed only 20% of the sulfadiazine, while ozone and O_3/UV showed a much higher removal rate. However, it is noteworthy that, although an excellent degradation rate was obtained, the initial sulfonamide concentration studied was 25 mg L^{-1}, well above that investigated in this study.

In the study by Yang *et al.* (2016), biodegradation was used to remove three sulfonamides (sulfamethoxazole, sulfadimetoxin and sulfamethazine) with an initial concentration of 100 mg kg^{-1}. A removal rate of 41.7% was obtained. In the study by Liu & Wang (2013) the degradation of sulfamethazine with an initial concentration of 20 mg L^{-1} by gamma irradiation in the presence of hydrogen peroxide was investigated. This process was also efficient for the removal of this drug.

Thus, it can be noted that the studies in the literature refer to initial concentrations much higher than those found in nature.

When the ozonation assays were carried out in the TW matrix (Figure 1(a) and 1(b)), we noted that at pH 7.0 a higher ozone dose was required for the same degradation efficiency than at pH 3.0.

At pH 7.0, the maximum dose of ozone for a degradation >99% of the six sulfonamides was 24 mg L^{-1}. At pH 3.0, for the same dose of ozone, the degradation was >99% for SDA, >99% for all six antimicrobials. As verified for ultrapure water, higher doses of ozone were required at pH 11.0 (Figure 1(c)).

In this aqueous matrix it was also possible to obtain a correlation between the degradation rate of sulfonamides and caffeine at pH 3.0. That is, at this pH, degrading 80% of the caffeine indicator, a >99% elimination of sulfonamides is guaranteed.

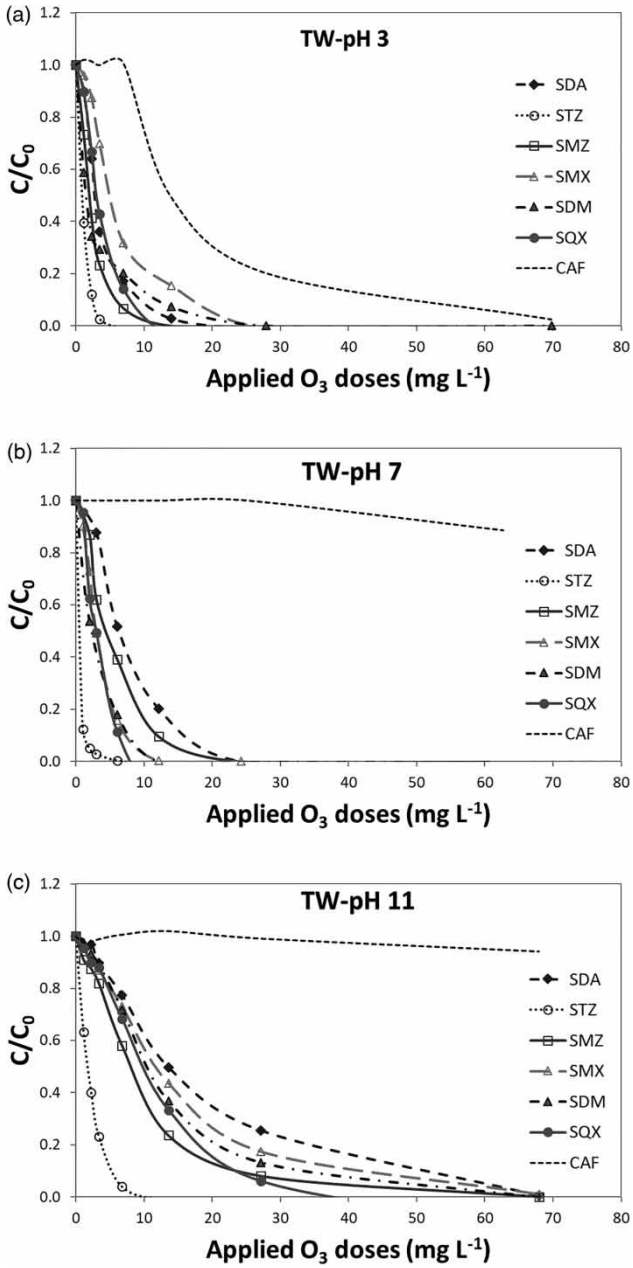

Figure 1 | Sulfonamide degradation using caffeine as indicator, in TW, at (a) pH 3.0, (b) pH 7.0, and (c) pH 11.0.

Regarding caffeine, it was noted that the degradation of this indicator became more difficult the higher the pH value.

The relevance of pH control during the degradation process must be emphasized, since the generation of carboxylic acids can reduce the pH value. In this study, no significant changes were noticed during the experiment, probably because of the low concentration level of sulfonamides in solution.

For SW, the degradation of the same antimicrobials required a similar applied ozone dose when compared with TW (Figure S2).

With the matrix of surface water at pH 3.0, degrading 69% of the caffeine is expected to remove over 99% of sulfonamides evaluated in this study. As for pH 11.0, it was necessary to degrade more than 34% of the indicator to achieve a 95% elimination rate of those antimicrobials. Finally, at pH 7.0, even if the sulfonamides had already been more than 99% degraded, no degradation of the caffeine was observed, and it is not possible to obtain a correlation between the antimicrobials and the indicator at this pH value.

As expected, when the ozonation was carried out using the ultrapure water matrix, better degradation was verified; this result can be explained by the fact that this matrix does not have hydroxyl radical scavengers, such as chloride, nitrite, bicarbonate and carbonate, among others. The other two aqueous matrices interfered in the degradation of the drugs due to these same radical scavengers. In addition, in SW, organic matter is present and oxidized by hydroxyl radicals. The results of this study are in accordance with Caianelo *et al.* (2017), who tested different matrices for the degradation of gatifloxacin.

At pH 11.0, with degradation >96.4% of the sulfonamides, there was only 8.9% of the degraded caffeine. Thus, for this matrix, one can verify how effective caffeine is as an indicator of correlation between the degradation of the compounds studied. Rosal *et al.* (2009) and Torun *et al.* (2014) studied caffeine degradation by ozonation although none of them focused on its use as a contamination indicator. After the degradations, the toxicity tests and an antimicrobial activity assay against Gram-positive (*B. subtilis*) and Gram-negative (*E. coli*) bacteria were performed.

In the toxicity test using the bacteria *Vibrio fischeri* (Figure S3), a slight increase in toxicity was noted over time to pH 11.0 and pH 7.0. At these pH values, the inhibition of luminescence increased from 0% to 51% (pH 7.0) and from 1% to 57% (pH 11.0) throughout the ozonation period. Nevertheless, at pH 3.0, the inhibition of luminescence was not significant, indicating that no intermediates with higher toxicity were formed. The different products formed during the reactions between the hydroxyl radicals and the six sulfonamides studied may penetrate more easily into the cell of the *V. fischeri* bacteria, resulting in the increase of solution toxicity (Urbano *et al.* 2017).

In the toxicity assays with *D. similis* (Figure S4), the results showed that, for the tap water matrix, the toxicity increased with the times at pH 7.0 and 11.0, and remained approximately constant at pH 3.0. For the matrix of surface water, no increased toxicity was found at the pH studied. An explanation for this would be that the surface matrix is a more favorable environment for the survival of *Daphnia similis*, so degradation products from the ozonation process would not substantially affect the mortality of these microcrustaceans. The graphical results of the toxicity assays and antimicrobial activity assays, which are detailed in the Supplementary Material, show the mean values of duplicates.

Studies on the toxicity of sulfonamides have been carried out (De Liguoro *et al.* 2009; Huang *et al.* 2014). Huang *et al.* (2014) emphasize that only the concentrations they used of 400 mg L^{-1} and 800 mg L^{-1} had a toxic effect on *Daphnia similis*. Therefore, the toxic effects are more difficult to conclude due to the very low initial concentration of this study (100 μg L^{-1}). De Liguoro *et al.* (2010) evaluated the toxicity of two sulfonamides using the *Daphnia magna* test organism. Although they obtained high survival rates from these microcrustaceans, the concentrations used in their study were also in the mg/L range.

Regarding antimicrobial assays (Figure S5), the results did not show a significant increase in bacterial growth inhibition. That can be explained by the low concentration imposed on these tests and the fact that the sulfonamides do not present very toxic behavior to the bacteria used in this study (*E. coli* and *B. subtilis*).

The reaction of the sulfonamides with ozone can be adjusted by the well-established first-order kinetics and the rate constants (k) are shown in the Supplementary Material. As can be observed from Tables S6 to S11, the rate constant, k, increases with pH value decrease. This means that the sulfonamides are more easily degraded by molecular ozone (O_3) than hydroxyl radical ($^{\bullet}OH$). This kinetic behavior was observed by Urbano *et al.* (2017) during sulfaquinoxaline ozonation.

CONCLUSIONS

This study has found that ozonation at pH 3.0, especially via molecular ozone, was more effective on the degradation of

the six sulfonamides than when carried out at pH 11.0, via hydroxyl radicals. At all pHs studied, ozonation allowed the degradation of more than 99% of the initial concentration of the proposed drugs; however, under acidic conditions (pH 3.0), the ozone dose required was smaller.

Also, it was noted that water matrices affect the process efficiency, since the more complex the aqueous matrix, the greater the ozone dosage required to degrade the same as in simpler matrices such as ultrapure water.

Regarding toxicity assays using the marine bacteria *V. fischeri*, it was noted that the lowest toxicity presented occurred at pH 3.0. In the test involving the microcrustaceans *Daphnia similis*, it was verified that, for the tap water matrix, the highest toxicities occurred at pH 7.0 and 11.0, and the surface water matrix showed no significant toxicity. Although the current study is based on a few numbers of antimicrobials from the same class, the findings suggest that an even greater influence would be found, considering that most antimicrobials are still present in waters treated by conventional processes. These findings enhance our understanding that continuous investigations about efficient methods for the degradation of these antimicrobials are necessary.

Finally, the use of caffeine as a degradation indicator between the compounds was quite satisfactory, since its degradation happens more slowly than the sulfonamides studied herein. It is important to emphasize that caffeine is not a toxic compound, so it was not the concern of this study to eliminate it, but to relate it to the degradation of the sulfonamides studied. It is concluded, therefore, that if large amounts of caffeine were found using ozone, which is a powerful oxidant, one can imagine how many other compounds are not eliminated by using the traditional water treatment processes.

Thus, the use of caffeine as an indicator of the degradation level for several other compounds and processes deserves further study.

ACKNOWLEDGEMENTS

FAPESP (2013/09543-7 and 2013/07817-2), CNPq (459078/2014-3 and 479131/2013-9) and CAPES (scholarship Glenda Araujo Silva Gireli).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this paper is available online at https://dx.doi.org/10.2166/ws.2019.182.

REFERENCES

ABNT (Associação Brasileira de Normas Técnicas (Brazilian Association of Technical Standards)) 2009 *NBR 12713 – Aquatic Ecotoxicology – Acute Toxicity – Test with Daphnia spp (Cladocera, Crustacea)*. ABNT, Rio de Janeiro, Brazil.

APHA 2012 *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington, DC.

Caianelo, M., Rodrigues-Silva, C., Maniero, M. G. & Guimarães, J. R. 2017 Antimicrobial activity against Gram-positive and Gram-negative bacteria during gatifloxacin degradation by hydroxyl radicals. *Environmental Science and Pollution Research* **24**, 6288–6298.

Dal Bosco, S. M., Barbosa, I. M., Candello, F. P., Maniero, M. G., Rath, S. & Guimarães, J. R. 2011 Degradation of ivermectin by Fenton and photo-Fenton and toxicity test using *Daphnia similis*. *Journal of Advanced Oxidation Technologies* **14**, 292–301.

De Liguoro, M., Fioretto, B., Poltronieri, C. & Gallina, G. 2009 The toxicity of sulfamethazine to *Daphnia magna* and its additivity to other veterinary sulfonamides and trimethoprim. *Chemosphere* **75**, 1519–1524.

De Liguoro, M., Di Leva, V., Gallina, G., Faccio, E., Pinto, G. & Pollio, A. 2010 Evaluation of the aquatic toxicity of two veterinary sulfonamides using five test organisms. *Chemosphere* **81**, 788–793.

Environmental Protection Agency (EPA) 1999 *Alternative Disinfectants and Oxidants Guidance Manual*, EPA 815-R-99-014. USEPA, Washington, DC, USA. Available from: http://www.epa.gov/ogwdw/mdbp/alternative_disinfectants_guidance.pdf (accessed 5 April 2018).

Food and Agriculture Organization of the United Nations (FAO) 2016 *The FAO Action Plan on Antimicrobial Resistance 2016–2020*. Food and Agriculture Organization of the United Nations, Rome, Italy.

García-Galán, M. J., Garrido, T., Fraile, J., Ginebreda, A., Díaz-Cruz, M. S. & Barceló, D. 2011 Application of fully automated online solid phase extraction–liquid chromatography–electrospray–tandem mass spectrometry for the determination of sulfonamides and their acetylated metabolites in groundwater. *Analytical and Bioanalytical Chemistry* **399**, 795–806.

Garoma, T., Umamaheshwar, S. K. & Mumper, A. 2010 Removal of sulfadiazine, sulfamethizole, sulfamethoxazole and sulfathiazole from aqueous solution by ozonation. *Chemosphere* **79**, 814–820.

Göbel, A., McArdell, C. S., Joss, A., Siegrist, H. & Giger, W. 2007 Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. *Science of The Total Environment* **372**, 361–371.

Guimarães, J. R., Barbosa, I. M., Maniero, M. G. & Rath, S. 2014 Abamectin degradation by advanced oxidation processes: evaluation of toxicity reduction using *Daphnia similis*. *Journal of Advanced Oxidation Technologies* **17**, 82–92.

Guo, W., Yang, Z., Du, J., Yin, R., Zhou, X., Jin, S. & Ren, N. 2016 Degradation of sulfadiazine in water by a UV/O_3 process: performance and degradation pathway. *RSC Advances* **6**, 57138–57143.

Homem, V. & Santos, L. 2011 Degradation and removal methods of antibiotics from aqueous matrices – a review. *Journal of Environmental Management* **92**, 2304–2347.

Huang, D., Hou, J., Kuo, T. & Lai, H. 2014 Toxicity of the veterinary sulfonamide antibiotic sulfamonomethoxine to five aquatic organisms. *Environmental Toxicology and Pharmacology* **38**, 874–880.

Kim, H., Hong, Y., Park, J., Sharma, V. K. & Cho, S. 2013 Sulfonamides and tetracyclines in livestock wastewater. *Chemosphere* **91**, 888–894.

Lin, A. Y. C., Lin, C. F., Chiou, J. M. & Hong, P. K. A. 2009 O_3 and O_3/H_2O_2 treatment of sulfonamide and macrolide antibiotics in wastewater. *Journal of Hazardous Materials* **171**, 452–458.

Liu, Y. & Wang, J. 2013 Degradation of sulfamethazine by gamma irradiation in the presence of hydrogen peroxide. *Journal of Hazardous Materials* **250–251**, 99–105.

Paíga, P. & Delerue-Matos, C. 2017 Anthropogenic contamination of Portuguese coastal waters during the bathing season: assessment using caffeine as a chemical marker. *Marine Pollution Bulletin* **120**, 355–363.

Petrie, B., Barden, R. & Kasprzyk-Hordern, B. 2015 A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. *Water Research* **72**, 3–27.

Rosal, R., Rodríguez, A., Perdigón-Melón, J. A., Petre, A., García-Calvo, E., Gómez, M. J., Agüera, A. & Fernández-Alba, A. R. 2009 Degradation of caffeine and identification of the transformation products generated by ozonation. *Chemosphere* **74**, 825–831.

Sui, M. H., Xing, S. C., Zhu, C. Y., Sheng, L., Lu, K. X. & Gao, N. Y. 2011 Kinetics of ozonation of typical sulfonamides in water. *Biomedical and Environmental Sciences* **24**, 255–260.

Torun, M., Abbasova, D., Şolpan, D. & Güven, O. 2014 Caffeine degradation in water by gamma irradiation, ozonation and ozonation/gamma irradiation. *Nukleonika* **59**, 25–35.

Urbano, V. R., Maniero, M. G., Pérez-Moya, M. & Guimarães, J. R. 2017 Influence of pH and ozone dose on sulfaquinoxaline ozonation. *Journal of Environmental Management* **195**, 224–231.

van Leeuwen, J. 2015 Proposed OS&E requirement: measuring ozone dosage. *Ozone: Science & Engineering* **37**, 191–192.

Won, J. S., Lim, D. H. & Seo, G. T. 2014 Degradation of sulfonamide antibiotic substances by ozonation: an experimental and computational approach. *Journal of Korean Society of Environmental Engineers* **36**, 442–450.

Wu, J. T., Wu, C. H., Liu, C. Y. & Huang, W. J. 2015 Photodegradation of sulphonamide antimicrobial compounds (sulfadiazine, sulfamethiazole, sulfamethoxazole and sulfathiazole) in various UV/oxidant systems. *Water Science and Technology* **71**, 412–417.

Wu, Q., Li, S., Zhao, X. & Zhao, X. 2018 Interaction between typical sulphonamides and bacterial diversity in drinking water. *Journal of Water & Health* **16**, 914–920.

Xu, W., Zhang, G., Zheng, M., Chen, J. & Wang, K. 2010 Treatment of antibiotic wastewater by ozonation. *Progress in Chemistry* **22**, 1002–1009.

Yang, C., Hsiao, W. & Chang, B. 2016 Biodegradation of sulfonamide antibiotics in sludge. *Chemosphere* **150**, 559–565.

Yu, H., Ge, P., Chen, J., Xie, H. & Luo, Y. 2017 The degradation mechanism of sulfamethoxazole under ozonation: a DFT study. *Environmental Science: Processes and Impacts* **19**, 379–387.

First received 18 February 2019; accepted in revised form 20 November 2019. Available online 6 December 2019